The Th2 Systemic Immune Milieu Enhances Cutaneous Inflammation in the K14-IL-4-Transgenic Atopic Dermatitis Model

Journal of Investigative Dermatology (2011) **131,** 791–794; doi:10.1038/jid.2010.382; published online 30 December 2010

TO THE EDITOR

Cutaneous immune responses are affected by the systemic immune milieu. Infection by *Mycobacterium leprae* produces the dichotomous tuberculoid and lepromatous forms of immune responses in a T helper type 1 (Th1)-dominant and a Th2-dominant milieu, respectively (Yamamura *et al.*, 1991). In animal models, cutaneous *Leishmania major* infection is resistant by Th1-dominant C57BL/6 mice but is susceptible in Th2-dominant BALB/c mice (Lopez-Kostka *et al.*, 2009). Interestingly, atopic dermatitis (AD), a chronic inflammatory skin disease, also manifests in various degrees of clinical severity (Jones *et al.*, 1975; Bonness and Bieber, 2007; Hanifin, 2009). We have previously generated a mouse model of AD by overexpression of a Th2 cytokine IL-4 in the basal epidermis of transgenic (Tg) mice (Chan *et al.*, 2001). Using this model, we documented that the disease onset occurs in parallel with a surge of cutaneous Th2 cytokines (Chen *et al.*, 2004). However, the extent to which a Th2-dominant systemic immune milieu influences the cutaneous inflammation is unknown. Therefore, this study was conducted to answer this question.

We crossed and backcrossed the original IL-4-Tg mice of the outbred CByB6 strain into purebred Th1-dominant C57BL/6 and Th2-dominant BALB/c strains (Harlan, Indianapolis, IN), generating IL-4-Tg C57BL/6-Tg (C-Tg) and IL-4-Tg BALB/c-Tg (B-Tg) strains. All generations of these mice were housed in special pathogen-free cages and fed regular mouse chow and standard water. Owing to heavy disease burden and inability to breed further, only three generations were obtained in the BALB/c strain. Therefore, seventh-generation C-Tg and third-generation B-Tg mice were studied (as approved by the University of Illinois at Chicago Animal Care Committee). Mice were killed 3 weeks after dermatitis onset, and sera and skin lesions were collected. For nontransgenic (NT) littermate controls, sera and normal ear tissues were collected from age-matched C57BL/6-NT (C-NT) and BALB/c-NT (B-NT) offspring. We compared the clinical phenotypes of these Tg strains, including age of onset and percentage of skin affected. We determined relative skin cytokine mRNA quantities by real-time PCR following reverse transcription of skin-extracted RNA (Chen *et al.*, 2004), total sera IgE, and IgG1 (by sandwich ELISA) (Chen *et al.*, 2005b), and skin inflammatory cells/vascular markers/adhesion molecules were quantified by immunofluorescence microscopy (Chen *et al.*, 2005a). Statistical analyses were performed using GraphPad Instat Software (GraphPad, San Diego, CA). The difference between medians of two groups was determined by the Mann–Whitney test. Experimental data were processed as means ± SEM. A *P*-value of $\leqslant 0.05$ was considered significant.

As expected, skin lesions characteristic of AD were observed in all C-Tg ($n = 12$) and B-Tg ($n = 11$) mice, but in none of the NT mice (Figure 1a and b) (Chen *et al.*, 2004). Skin lesions were clinically similar, with erythema, scaling, and crusting (Figure 1a). Histopathologically, hematoxylin and eosin staining showed similar morphology of acanthosis, hyperkeratosis, parakeratosis, spongiosis, and prominent inflammatory infiltrates in both Tg strains (Figure 1b). Both Tg mouse strains developed skin inflammation notwithstanding that significantly more body surface area was affected in the B-Tg mice than in the C-Tg mice ($P < 0.001$, Figure 1a and c), and the B-Tg mice developed dermatitis much earlier than did C-Tg mice ($P < 0.0001$, Figure 1d). These data support a notion that a Th2-dominant background enhances the clinical features of AD.

We next examined whether total serum IgE levels affect skin inflammation. Although C-Tg mice had a statistically significant higher IgE level than did C-NT mice, B-Tg mice did not increase their IgE levels relative to B-NT mice, whose baseline IgE levels were higher than those in C-NT mice (Figure 1e). The IgG1 level in B-NT mice (average 3.48 ng ml^{-1}) was also significantly higher than that in C-NT mice (average 1.33 ng ml^{-1}, $P < 0.05$). Although the absence of IgE upregulation is known in the intrinsic subset of AD (Novak and Bieber, 2003) and in the SKH1-IL-4-Tg AD model (Chen *et al.*, 2008b), the mechanism of IgE regulation in B-Tg mice remains unclear.

We then asked whether cytokine levels in skin lesions of Th2-dominant B-Tg mice (earlier onset with higher percentage of skin affected) differ from those of Th1-dominant C-Tg mice (later onset with smaller percentage of skin affected). Significantly greater numbers of mRNA copies of Th2 cytokines (IL-4, IL-10, IL-13), proinflammatory cytokine IL-1β, and Th1 cytokine IL-2 were found in the lesional skin of B-Tg mice than in that of C-Tg mice ($P < 0.05$, Figure 1f). IL-6, INF-γ, and tumor necrosis factor-β levels were higher in B-Tg mice than in C-Tg mice,

Abbreviations: AD, atopic dermatitis; B-Tg, BALB/c-transgenic; C-NT, C57BL/6-nontransgenic; C-Tg, C57BL/6-transgenic; NT, nontransgenic; Tg, transgenic

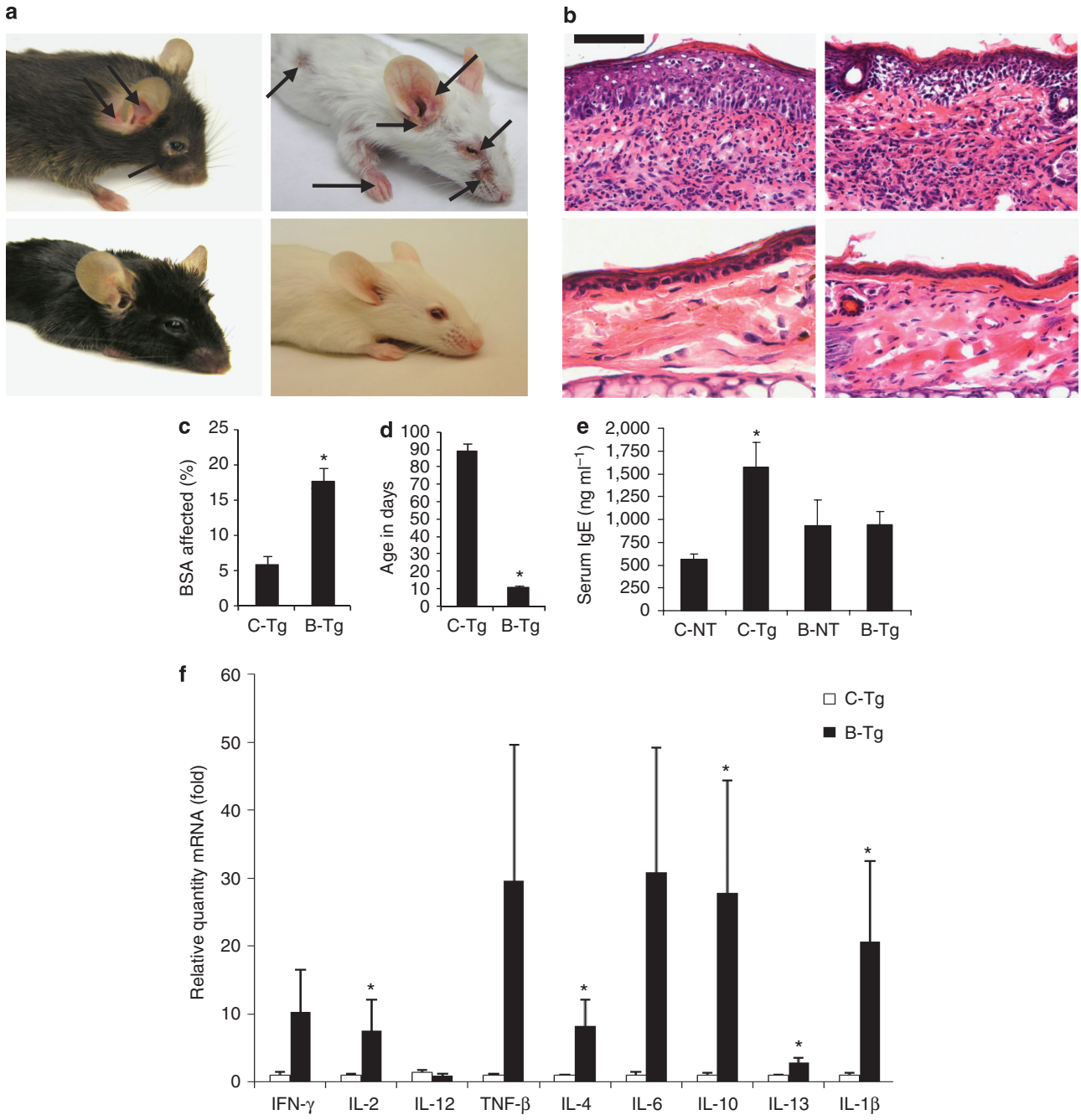
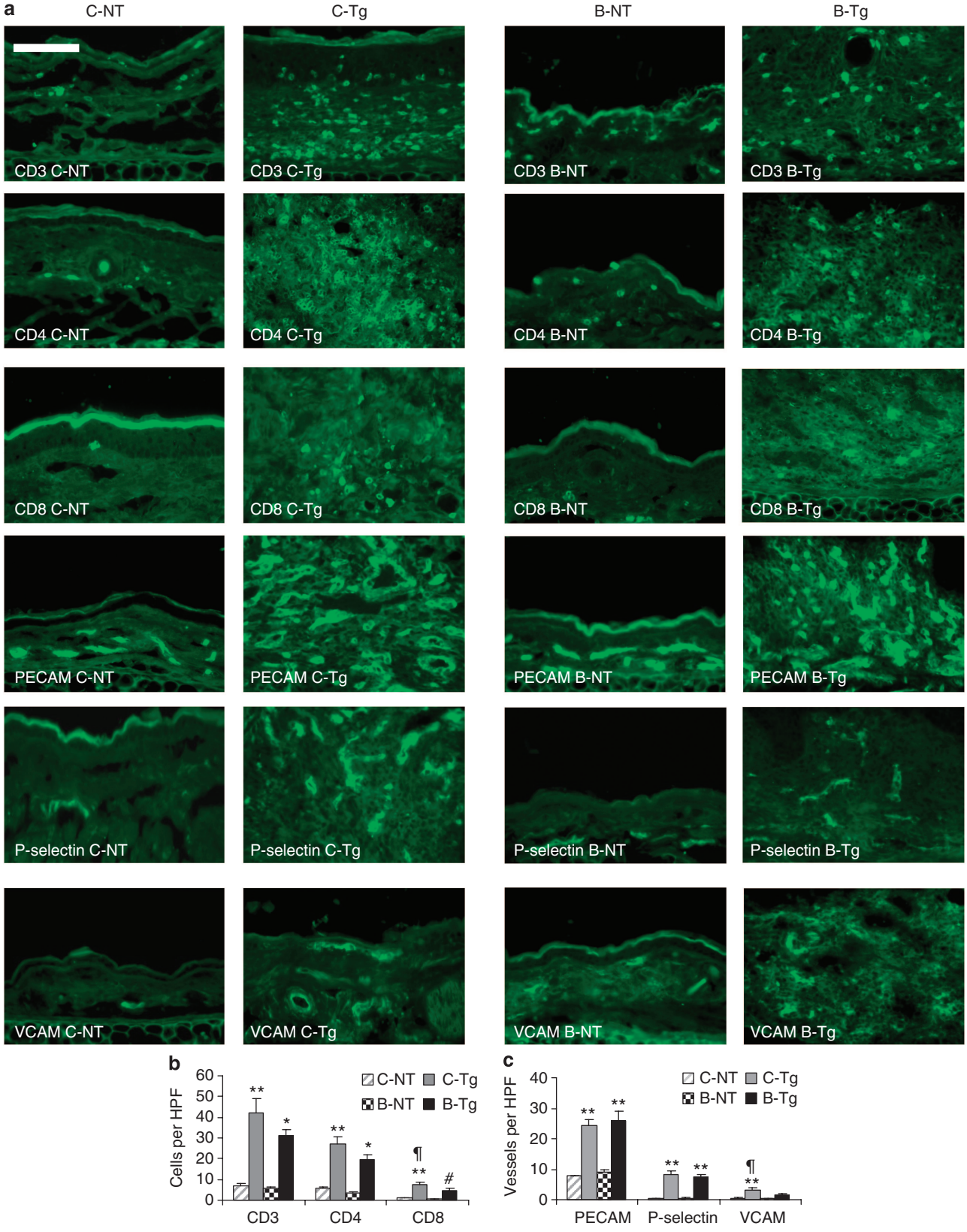


Figure 1. B-Tg mice developed skin lesions earlier and more extensively and exhibited higher levels of Th2 cytokines and other pro-inflammatory cytokines than did C-Tg mice. (**a**) B-Tg mice (top right) had more lesions compared with C-Tg mice (top left), whereas no lesions were seen in in C-NT mice (bottom left) and B-NT mice (right bottom). (**b**) Both C-Tg (top left) and B-Tg (top right) skin lesions showed acanthosis, hyperkeratosis, parakeratosis, spongiosis, and inflammatory infiltrates compared with the corresponding NT histology (C-NT bottom left; B-NT bottom right). H&E, ×40. Bar = 70 μm. (**c**) B-Tg mice had significantly more body surface area affected (17.7 ± 1.8%) than did C-Tg mice (5.8 ± 1.3%), $*P<0.001$. (**d**) In B-Tg mice inflammation onset occurred at a significantly younger age (11.0 ± 2.0 days) than in C-Tg mice (89.1 ± 4.6 days), $*P<0.0001$. (**e**) Total serum IgE significantly increased in C-Tg (1,574.6 ± 269.37) versus C-NT (567.58 ± 57.20) mice, $*P<0.05$. (**f**) B-Tg mice had higher levels of nearly all cytokines than did C-Tg mice. $*P<0.05$. B-NT, BALB/c-nontransgenic; B-Tg, BALB/c-transgenic; C-NT, C57BL/6-nontransgenic; C-Tg, C57BL/6-transgenic; H&E, hematoxylin and eosin.

Figure 2. B-Tg and C-Tg mice exhibited similar infiltrating T cells, vasculature, and vascular adhesion molecules. (**a**) Immunofluorescence microscopy with primary antibodies and Alexa fluor 488-conjugated secondary antibodies. Bar = 70 μm. (**b**, **c**) Dermal numbers per high-power field (HPF) for C-NT, C-Tg, B-NT, and B-Tg mice are CD3+ (7.19 ± 0.91, 42.01 ± 6.74, 5.67 ± 0.52, 31.27 ± 3.05, respectively), CD4+ (5.69 ± 0.53, 26.86 ± 3.80, 3.42 ± 0.37, 19.42 ± 2.23), CD8+ (0.89 ± 0.24, 7.22 ± 1.22, 0.67 ± 0.14, 4.83 ± 0.80), PECAM+ (7.67 ± 0.36, 24.36 ± 1.76, 9.21 ± 0.53, 26.07 ± 2.82), P-selectin+ (0.24 ± 0.09, 8.20 ± 1.02, 0.49 ± 0.20, 7.44 ± 0.86), and VCAM+ (0.54 ± 0.14, 3.26 ± 0.57, 0.31 ± 0.12, 1.72 ± 0.19). Significant as compared with the NT strain: $^{\#}P<0.05$, $*P<0.01$, $**P<0.001$. Significant comparison between C-Tg and B-Tg strains: $^{¶}P<0.05$. B-NT, BALB/c-nontransgenic; B-Tg, BALB/c-transgenic; C-NT, C57BL/6-nontransgenic; C-Tg, C57BL/6-transgenic.

although not significantly. Our data are consistent with findings in another Tg murine AD model that higher levels of Th2 cytokines correlated with earlier development and increased severity of AD (Lee and Flavell, 2004).

We further investigated whether enhanced skin inflammation in B-Tg mice can be explained by variations in



lesional inflammatory cell infiltration, vascular adhesion molecules, or angiogenesis (Figure 2). When comparing C-Tg with C-NT mice, we found significantly more CD3+ cells, CD4+ cells, and CD8+ cells in the Tg strain's dermis (all with $P<0.001$). Similarly, the B-Tg dermis had significantly more CD3+ cells ($P<0.01$), CD4+ cells ($P<0.01$), and CD8+ cells ($P<0.05$) compared with its B-NT counterpart. Both C-Tg and B-Tg mice had significantly more PECAM+ vessels per high-power field compared with their NT littermates ($P<0.001$), further supporting a role of angiogenesis in AD (Chen *et al.*, 2008a). Similarly, C-Tg and B-Tg mice had more P-selectin+ and VCAM+ vessels than did their NT littermates ($P<0.001$); this was identical to the previous findings in AD patients and in an AD model (Sigurdsson *et al.*, 2000; Chen *et al.*, 2010). There was no difference in numbers of CD3+ and CD4+ lymphocytes, PECAM+, and P-selectin+ vessels between the dermis of the two Tg strains. The physiological implications of more CD8+ cells and VCAM+ vessels per high-power field in the dermis but with less skin inflammation in C-Tg mice, as compared with B-Tg mice, are unclear (Figure 2b and c).

The Th2 systemic immune milieu strongly enhances the rate and extent of cutaneous inflammation in our AD model by primarily increasing the expression of cutaneous Th2 cytokines and other proinflammatory cytokine.

CONFLICT OF INTEREST

The authors state no conflict of interest.

ACKNOWLEDGMENTS

This work has been supported by the Albert H. and Mary Jane Slepyn Endowed Fellowship, University of Illinois College of Medicine, Chicago, Illinois.

Marie E. Reichle[1], Lin Chen[2], Shao-Xia Lin[1] and Lawrence S. Chan[1,3,4]

[1]Department of Dermatology, College of Medicine, University of Illinois at Chicago, Chicago, Illinois, USA; [2]Department of Oral Biology, College of Dentistry, University of Illinois at Chicago, Chicago, Illinois, USA; [3]Department of Microbiology/Immunology, College of Medicine, University of Illinois at Chicago, Chicago, Illinois, USA and [4]Medicine Service, Jesse Brown VA Medical Center, Chicago, Illinois, USA
E-mail: larrycha@uic.edu

REFERENCES

Bonness S, Bieber T (2007) Molecular basis of atopic dermatitis. *Curr Opin Allergy Clin Immunol* 7:382–6

Chan LS, Robinson N, Xu L (2001) Expression of interleukin-4 in the epidermis of transgenic mice results in a pruritic inflammatory skin disease: an experimental animal model to study atopic dermatitis. *J Invest Dermatol* 117:977–83

Chen L, Lin S-X, Amin S *et al.* (2010) VCAM-1 blockade delays disease onset, reduces disease severity and inflammatory cells in an atopic dermatitis model. *Immunol Cell Biol* 88:334–42

Chen L, Lin SX, Overbergh L *et al.* (2005b) The disease progression in the keratin 14 IL-4-transgenic mouse model of atopic dermatitis parallels the up-regulation of B cell activation molecules, proliferation and surface and serum IgE. *Clin Exp Immunol* 142:21–30

Chen L, Marble DJ, Agha R *et al.* (2008a) The progression of inflammation parallels the dermal angiogenesis in a keratin 14 IL-4-transgenic model of atopic dermatitis. *Microcirculation* 15:49–64

Chen L, Martinez O, Overbergh L *et al.* (2004) Early up-regulation of Th2 cytokines and late surge of Th1 cytokines in an atopic dermatitis model. *Clin Exp Immunol* 138:375–83

Chen L, Martinez O, Venkataramani P *et al.* (2005a) Correlation of disease evolution with progressive inflammatory cell activation and migration in the IL-4 transgenic mouse model of atopic dermatitis. *Clin Exp Immunol* 139:189–201

Chen L, Overbergh L, Mathieu C *et al.* (2008b) The development of atopic dermatitis is independent of immunoglobulin E up-regulation in the k14-IL-4 SKH1 mouse model. *Clin Exp Allergy* 38:1367–80

Hanifin JM (2009) Evolving concepts of pathogenesis of atopic dermatitis and other eczemas. *J Invest Dermatol* 129:320–2

Jones HE, Inouye JC, McGerity JL *et al.* (1975) Atopic disease and serum immunoglobulin-E. *Br J Dermatol* 92:17–25

Lee GR, Flavell RA (2004) Transgenic mice which overproduce Th2 cytokines develop spontaneous atopic dermatitis and asthma. *Int Immunol* 16:1155–60

Lopez-Kostka S, Dinges S, Griewank K *et al.* (2009) IL-17 promotes progression of cutaneous leishmaniasis in susceptible mice. *J Immunol* 182:3039–46

Novak N, Bieber T (2003) Allergic and nonallergic forms of atopic diseases. *J Allergy Clin Immunol* 112:252–62

Sigurdsson V, de Vries IJ, Toonstra J *et al.* (2000) Expression of VCAM-1, ICAM-1, E-selectin, and P-selectin on endothelium *in situ* in patients with erythroderma, mycosis fungoides and atopic dermatitis. *J Cutan Pathol* 27:436–40

Yamamura M, Uyemura K, Keans RJ *et al.* (1991) Defining protective responses to pathogens: cytokine profiles in leprosy lesions. *Science* 254:277–9

High Levels of Soluble ST2 and Low Levels of IL-33 in Sera of Patients with HIV Infection

Journal of Investigative Dermatology (2011) **131,** 794–796; doi:10.1038/jid.2010.366; published online 9 December 2010

TO THE EDITOR

IL-33, a novel member of the IL-1 family, was recently identified as a ligand for the orphan receptor ST2 (Schmitz *et al.*, 2005). ST2 is selectively and stably expressed on the cell surface of T-helper 2 (Th2) cells, but not Th1 cells (Xu *et al.*, 1998). IL-33–ST2 interactions exacerbate Th2- and mast cell-mediated inflammatory diseases (Préfontaine *et al.*, 2009). The soluble form of ST2 functions as a decoy

Abbreviations: AD, atopic dermatitis; Th, T helper